BASIC SCIENCES RESEARCH

Initial Interaction of the P22 Phage with the *Salmonella typhimurium* Surface

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Objectives. **The goals of these studies were to characterize the interaction of the P22 phage particle with the Salmonella cell surface and to determine the phage elements involved in this interaction by mutational analysis.**

Background. **The phage P22 has been characterized extensively. The gene and protein for the phage P22 tailspike, which is the phage adsorption organelle, have been intensively studied. The kinetics of the interaction of the tailspike protein with the cell surface has been studied in detail, surprisingly no mutational analysis has ever been reported that has defined these components and their interaction between themselves and the cell surface. The main and perhaps only component needed for this cell surface interaction is the tailspike protein.**

Methods. **Adsorption to the cell surface has been measured in the wild type phage and in mutant derivatives, isolated in this study. Phage mutants have been isolated after hydroxylamine mutagenesis.**

Results. **The adsorption of P22 to the cell surface is a temperature-independent event. Forty putative phage adsorption mutants have been isolated. A sample of them have been further analyzed. These divide the adsorption process into at least two stages. One stage contains mutants that absorb with essential wild type phage kinetics to the cell surface while the other stage with delayed adsorption kinetics.**

Conclusions. **The interaction of the phage P22 with the Salmonella cell surface has been shown to be a complicated one which is temperature-independent and multi-stage. Mutants isolated in this study may help dissect this process even further.**

Key words: Lipopolysaccharides(LPS)-protein interactions, Mutagenesis, P22 phage, Phage adsorption, Phage mutants, Tailspike protein, Salmonella typhimurium.

In recent times, there has been a dramatic and global surge in the number of antibiotic-resistant pathogenic microorganisms (1-3). This is in part responsible for the revival of interest in the study of bacterial viruses or phages. Phages have been shown to be intricately connected with infectious processes, which can carry genes for toxic agents such as cholera toxin. They have been directly and strongly linked to the formation and transduction of bacterial pathogenicity islands (4-6). Phages have been shown to be potentially useful as therapeutic intervention agents and a branch of viral studies dealing with phage therapy has developed (7-12). This report has ramifications in this area.

Most phages are confined to a narrow range in their selection of bacterial hosts to infect. These phages often show exquisite specificity in their choice of host and may be unable to infect a host because of a difference in the receptor of a single chemical group or bond. This specificity is especially evident in the *Salmonella* phages. Knowledge of how phages bind to the cell surface may allow the development of a bacteria-specific binding agent. Use is being made of a tail from a well-known virus as a model for these types of studies.

Many *Salmonella* phages have as an absolute requirement the interaction between their tails (or tailspike proteins, TSPs) and the host cell LPS during their infection cycle. The *Salmonella typhimurium* phage P22 is a prototype for this type of phage. It is the phage TSP which is responsible for the initial interaction leading to a productive infection (absorption) of the phage to the bacterial cell (13-16). The P22 TSP is the adhesin for the phage and is well characterized (17-19). The P22 TSP and its gene have been extensively characterized (20-22). The 3D structure consists predominantly of a ß-helix (398aa out of 666aa). The ß-helix is a ß-sheet protein structure, tracing out a helical pattern in space.

A genetic method is described for isolating phage mutants in the adsorption process (phage host range

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mutants). These mutants may yield insight into LPS binding by proteins. The data describes two types of adsorption mutants. These mutants may represent defects in two separate stages in the phage adsorption process. Binding characteristics of a small number of adsorption mutants are presented.

Methods

Bacterial strains. The *Salmonella enterica serovar* Typhimurium (hereafter *Salmonella typhimurium)* strains used in this study, TP104 and TP275, were derivatives of LT2 and were from our laboratory collection (originally gifts of A. Poteete, University of Massachusetts). Bacterial phage-resistant mutants, were derived as a byproduct in the process of isolation of phage host range mutants and are listed in Table 1.

Table 1. Salmonella strains used in this study.

Lab No.	Designation	Characteristic or properties	Source or Reference
BV4010	TP104	MS1017;leuAam414supO (su-)	A. Poteete
BV4012	TP275	leuAam414 supE (gln)	A. Poteete
BV4031	RS16	BV4012 P22^{r1}16or ptt16	this work
BV4036	RS21	BV4012 P22^{r} 21 or ptt21	this work
BV4042	RS27	BV4012 P22^{r} 27or ptt27	this work
BV4053	RS38	BV4012 P22^{r} 38or ptt38	this work
BV4069	RS54	BV4012 P22^{r} 54or ptt54	this work
BV4070	RS55	BV4012 P22^{r} 55or ptt55	this work
BV4090	RS75	BV4012 P22^{r} 75or ptt75	this work

Phage strains. The phage strains such as P22 *c1*-7 and P22 *c1*-7 *13am*H101 were from our laboratory stock collection and have been described (23). Phage stocks were prepared by the single plaque method as described (23). For the purposes of these studies these phage are wild type. Table 1 presents the phage strains obtained during the course of this investigation.

Adsorption. Cells were infected at a multiplicity of infection (MOI = ratio of the number of phage particles to the number of bacteria) of 1.0. At each five minute interval, for fifteen minutes, an aliquot of the infected cells was removed and centrifuged for 1 min at 14,000 x g in a microfuge. Phages that adsorbed to the bacteria were pelleted with the bacteria. The supernatant was decanted and the number of phage in the supernatant was determined. This is an indicator of the number of phages which did not bind to cells. From these data and the total input phage, the fraction of adsorbed phage was calculated as 1- (fraction unadsorbed). The percent adsorption is the ratio of the number of phages adsorbed over the total number of input phage. This fraction is then multiplied by one hundred. Studies were carried out at 30°C, unless otherwise indicated.

Isolation of phage mutants. A P22 *c*1-7 13amH101 phage stock was mutagenized, using the hydroxylamine (HA) protocol (24). Essentially, 10^{10} plaque forming units (pfu) of phages, were incubated in 0.4M HA for 48 hours at 37°C. Aliquots are withdrawn every 10h for 30h to monitor for the expected drop in phage titer due to the effect of the mutagen. Incubation of phage in HA for thirty hours diminished its concentration to 2% of an untreated control phage. The mutated phages were dialyzed and served as the phage stock for the isolation of phage host range mutants.

Selection for phage mutants with altered adsorption properties - isolation of phage host range mutants. To select for mutants with altered adsorption properties, phage host range mutants were isolated for the first time in P22 phage. To isolate host range mutants, first *Salmonella* cells are obtained that are resistant to the wild type P22 phage (RS strains), then P22 is mutagenized and phage are selected that can plate the Salmonella strains which are resistant to wild type P22 phage.

Salmonella typhimurium cells were infected with P22 *c1*-7 phage at a MOI of 20. The infected cell culture was mixed with molten agar, plated on a petri dish and incubated at 30°C for 16h. The surviving bacterial cells are resistant to wild type P22 phage particles. The RS mutant host cells were purified extensively, undergoing at least three rounds of single colony purification. One hundred such RS strains were isolated (denoted as RS1, RS2 etc). To prevent the formation of pseudolysogens, the RS mutant cells were infected with P22 *c1*-7. This phage can not form a lysogen. The culture supernatant was always checked for the presence of phage (characteristic of pseudolysogeny). A stock of P22 *c1*-7 phage particles was treated with HA, as described above. The mutant phage stock was used to separately infect each of the one hundred mutant RS host cells isolated. The mutant phages, forming plaques on individual RS strains, are defined as adsorption mutants. These phages were named in accordance with the Salmonella strain from which they could form plaques. Phage P22 (16-4) was the fourth phage isolated from RS16. These phage mutants serve as our adsorption mutants. These mutants are being characterized further.

Results

Interaction of wild type P22 TSP with *Salmonella typhimurium* LPS (Adsorption of wild type P22 to the bacterial surface). The ability of the P22 phage to adsorb

to *Salmonella typhimurium* host cells is a function of its tail or tailspike protein (TSP) and was measured under several conditions so that these data could serve as the baseline with which to compare the adsorption of putative mutants defective in this function.

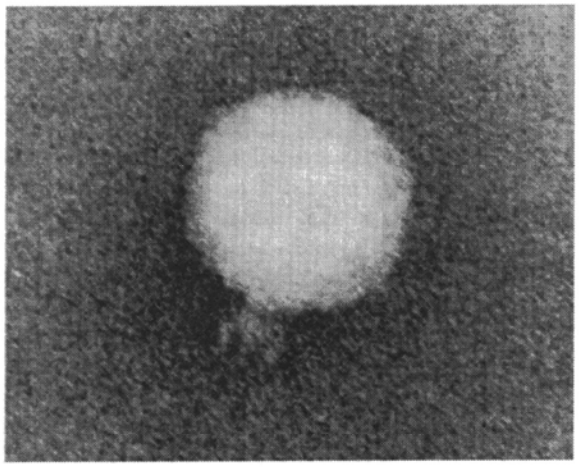

Figure 1. Electron micrograph of the *Salmonella typhimurium* phage P22 particle. The capsid length is about 64nm, and the tailspike length is about 6.4nm. The TSP is located at the bottom of the electron micrograph and appear as sharp projections from the phage (19).

To determine the effect of temperature on phage adsorption, bacterial cells were infected as described (Methods). Early sampling times were chosen because adsorption is complete by fifteen minutes after infection. These times points are too early in the phage life cycle to result in the production of additional phage particles. Phage production normally takes about one hour under these infection conditions (data not shown). Phage production would interfere with the adsorption calculations (Methods). Incubation of the infected cells was done at 23°C, 30°C, 37°C and adsorption was measured as described (Methods). Phage adsorption was also measured at 0°C, with very similar results. The data, in Figure 2, indicate that binding of the phage to the host cells was virtually complete within the first 5 min. Binding remained high at all temperatures tested, demonstrating that this process is independent of temperature.

Isolation of host range mutants of the phage P22. Phage host range mutants were isolated in two steps. Firstly, host cells resistant to wild type P22 were obtained by infecting cells with wild type P22 phage at an MOI (ratio of phage plaque forming units to bacterial cell) of twenty. The surviving host cell were resistant to infection by phage P22 and tested for the formation of pseudolysogens. These P22 resistant host cells formed at a frequency of about 1 x 10^{-8}. One hundred RS mutant host

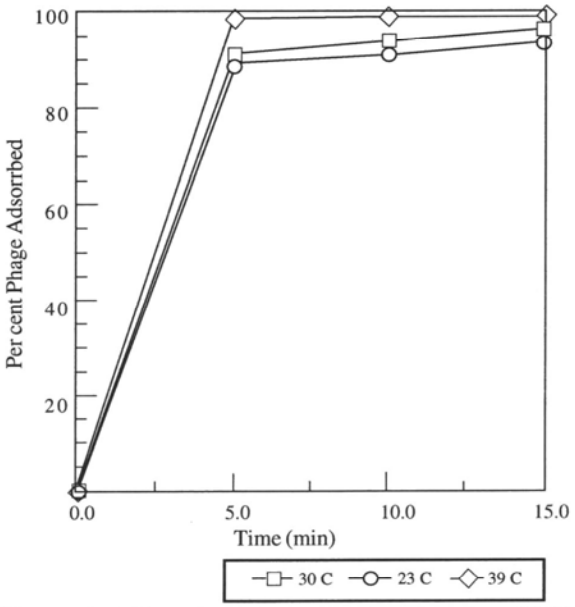

Figure 2. Effect of temperature on the adsorption of P22 phage onto *Salmonella typhimurium* cells. Adsorption was measured at three early time points after infection, five, ten and fifteen minutes, for each of the three temperatures tested: 23°C (open triangle), 30°C (open square) and at 39°C (open diamond).

strains were isolated and purified. All of these RS host cells were selected to be resistant to wild type P22 phage and control studies showed that binding of P22 phage to these cells was limited to 20% or less.

Secondly, to obtain a P22 host range mutant, wild type P22 phage was mutagenized with hydrolxylamine (HA) to a phage survival of 0.2% (Figure 3). The mutagenized

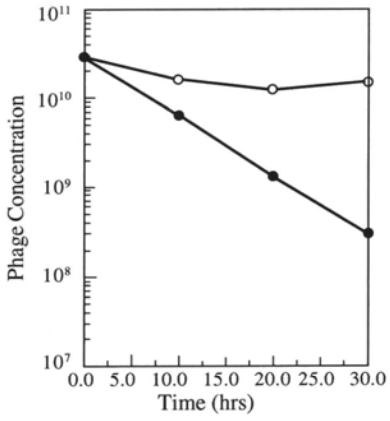

Figure 3. Hydroxylamine mutagenesis of P22 *13*amH101 phage for isolation of host range mutants of the phage. The untreated phage is represented by the empty squares while the phage treated with the mutagen is represented by the filled circles.

phage was used to infect the bacterial strains that were made resistant to wild type phage. Figure 4 shows the ability of one of the isolated phage (which was isolated BV4036 or RS21), P22 (21-12), to adsorb to the strain from which it was isolated (RS21) and it also shows how the wild type phage adsorbs very poorly on that strain. Forty putative adsorption mutants that could infect these resistant cells were isolated (Table 2).

Table 2. Phage Adsorption Mutants

bacterial Mutant	corresponding virus Mutant	No. of Mutants
RS16	P22 (16-4)	1
RS21	P22 (21-12)	1
RS27	P22 (27-48)	1
RS38	P22 (38-74A-I)	9
RS54	P22 (54-1->6; 9->17; 25->33)	23
RS55	P22 (55-1)	1
RS75	P22 (75-1A); (75 -1B, -2A, -2B)	4

To determine if the adsorption characteristics of these mutant phages had been changed, this property was determined for three phage mutants and for wild type phage at 30°C (Figure 5). The following phage mutants were tested: P22 (16-4), P22 (21-12), P22 (54-12) and wild type P22 phage (Table 2). These phage mutants share the ability to adsorb to mutant bacteria onto which wild type P22 phage binds very poorly (Figure 4). The binding was measured in the respective mutant host for each mutant phage (i.e. adsorption of phage P22 (16-4) was measured on RS16 (Table 1).

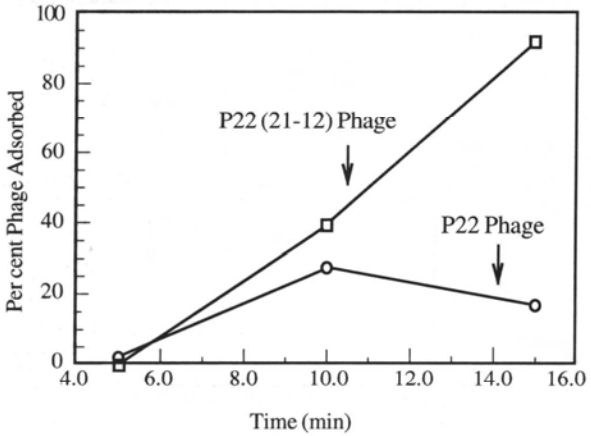

Figure 4. RS mutant cells do not adsorb wild type P22 phage well. Wild type phage P22 (open circles) and mutant phage P22 (21-12), (open squares) are adsorbed onto the surface of *Salmonella typhimurium* mutant host RS21 at 30°C.

The fact that there are two different patterns of phage adsorption, can be garnered from a comparison of Figures 2, 5 and 6. In Figure 5 it is apparent that these three phage mutants bind very poorly to the surface of their respective host cells, requiring at least ten minutes to attain noticeable adsorption. Two putative phage mutants, P22 (16-4) and P22 (54-12), did not reach wild type levels of adsorption even after fifteen minutes of interaction with their host cells. The second adsorption pattern is illustrated by the adsorption of phage P22 (75-1A) to its cognate host cell (Figure 6 and Table 2). Initially, this phage mutant displays a pattern of adsorption similar to that of the wild type phage, P22, on its normal host, BV4012 during initial adsorption at five minutes. Incubation of this phage mutant, P22 (75-1A), at different temperatures revealed that it does not display a uniformly tight binding adsorption phenotype at all temperatures. unlike wild type phage on wild type host cells (compare Figures 5 and 6).

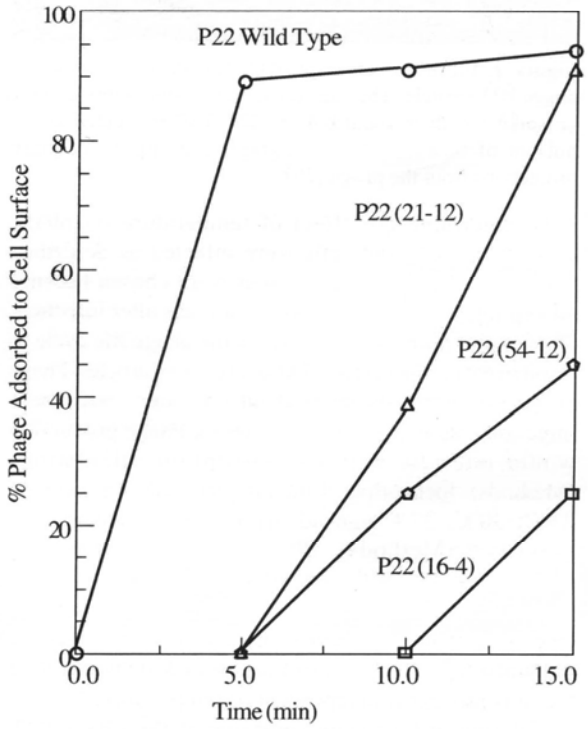

Figure 5. The adsorption characteristics of P22 phage mutants at 30°C. Adsorption was measured at three early time points after infection, five, ten and fifteen minutes, for four phage mutants and wild type phage at 30°C. Wild type phage is designated by the open circle, phage mutant P22 (75-1A) by the filled circle, phage mutant P22 (21-12) by the open triangle, phage mutant P22 (54-12) by the open diamond and phage mutant P22 (16-4) by open square.

with the phage tail protein and it has often been shown to be reversible. The data, reported here, indicate that the binding is very rapid and not dependent on temperature. These data are similar to that obtained from studies on the adsorption of *Salmonella anatum* phage ε^{15} and on *Salmonella newington* phage ε^{34} (30-31). The rapid binding kinetics most likely indicates an interaction of the P22 TSP with the host cell LPS.

However, hydrolysis has been measured to be very slow. It is in the order of two cleavages per minute (17). The discrepancy between the low enzymatic activity and the fast binding kinetics suggests that additional factors are present in the intact phage particle. Examples of such factors include: restriction of orientation of the P22 TSP relative to its substrate and the normally longer O-antigen repeat units present in the intact cell, may help accelerate the reaction *in vivo* as opposed to the purified *in vitro* system in which the kinetics was measured. Other studies have shown that catalytically-defective mutants of the P22 TSP bind LPS with essentially normal kinetics and binding affinity (17). This indicates that TSP binding to LPS and TSP catalytic activity can be functionally separate processes.

It is proposed that the mutants in which adsorption is altered may be defective in the ability of the TSP to bind to the LPS or in a late process such as that involving injection of the phage DNA into the host or any combination of these steps. Further studies should elucidate the causes of these defects.

Resumen

Este informe presenta la descripcion del primer aislamiento y caracterización de mutantes del fago P22 que son defectuosos en su absorción con la superficie de *Salmonella enterica* serovar *Typhimurium*. Estos estudios demuestran que la absorción del fago a las células de la bacteria es independiente a la temperatura. Las características del enlace entre el fago y las células ha sido usadas como una clasificación preliminar. El análisis de estos mutantes puede que nos provea ayuda para descifrar este proceso complejo de los pasos preliminares de la infección del fago P22.

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